Page 6, lines 8-9:

A1

Figure 3 is an image taken from an optical microscope in reflected light mode of a 9-striped bar code (Au/Ag/Au/Ag/Au/Ag/Au/Ag/Au) of the present invention.

Page 6, lines 19-20:

A2

Figure 6A is an image of a collection of Ag/Au nanorods at 400 nm and Figure 6B is an image of the same collection at 600 nm.

Page 9, lines 22-27:

A3

The particles of the invention are frequently referred to as being "rod" shaped. However, the cross-sectional shape of the particles, viewed along the long axis, can have any shape, and can change at different portions of the particle. Such cross-sections may be a circle, an oval, square, diamond or even tubular. In preferred embodiments of the invention, the cross section is a circle and the particles are "rod" shaped. Although the particles of the present invention may take many shapes, the segmented particles of the present invention are not spherical.

Page 16, lines 17-29:

A4

A key property of certain embodiments of the particles of the present invention is that when the nanorods are segmented, differences in the reflectivities of the component metals can be visualized by optical microscopy. Thus, in a segmented Au/Pt/Au rod of 200 nm in diameter and 4-5 microns in overall length, the segments are easily visualized in a conventional optical microscope, with the Au segments having a gold lustre, and the Pt segments having a more whitish, bright lustre. Another key property of the materials is that the length of the segments, when they are prepared by alternating electrochemical reduction of two or more metal ions, is controlled (and defined) completely by a) the composition of the solution and b) the number of Coulombs of charge that are passed in each step of an electrochemical reduction. Thus, the widths and the number of the segments can be varied at will. Figure 5 shows an image of a collection of nanoparticles of the present invention comprised of six different types or flavors of nanoparticles. This

image demonstrates the ability to differentiate between the different types of nanobar codes in a collection of nanobar codes.

A4 Cont'd

Page 18, lines 9-14:

A unique characteristic of nanobar codes, is the ability to differentially modify their surfaces. Thus, considering Au/Pt nanobar codes, each metal may be selectively modified, providing two different chemistries to be placed in close proximity. In the case of nanobar codes, the ability to put different molecules on the Pt and Au stripes of a single particle has been demonstrated. The ability to rationally modify selected parts of a nanoparticle is without precedent.

A5

Page 23, line 21:

A6

PREPARATION OF METALLIC SEGMENTED PARTICLES

Page 24, lines 13-21:

A7

To generate two-segment nanobar codes, two metals (e.g., Au, Ag, Pd, Cu, etc.) can be electroplated sequentially or simultaneously to form alloys. Nanobar codes can also be generated using 3 different metals. Synthesis of a Au/Pt/Au rod may be accomplished with 1 C of Au, 8 C Pt, and 1 C of Au. The nominal dimensions of the segments are 1 μm of Au, 3 μm of Pt, 1 μm of Au. The 5-segment nanobar codes, Ag/Au/Ag/Au/Ag, were generated by sequentially plating and optionally rinsing the appropriate metal. In some embodiments it is possible to include all metals in solution but control deposition by varying the charge potential current A nine-segment nanobar code, Au/Ag/Au/Ag/Au/Ag/Au/Ag/Au seen in Figure 3 has also been prepared. The number of segments can be altered to desired specifications.

Page 28, lines 12-21:

A8

The ability to make complex bar codes is of no consequence without an effective method for reading the bar codes. Fortunately, in the case of metallic bar codes of approximately 100 nm or more in width and about 2 microns to 15 microns in length, differences in metal segment reflectivities can be visualized using conventional light microscopy. Thus, is

possible to distinguish (and quantify) the number of rods by visual inspection; such a task could be automated. It is also possible to distinguish segments of different lengths within individual bar codes. Images have been obtained of a 9-striped bar code (Au/Ag/Au/Ag/Au/Ag/Au/Ag/Au) in which the four Ag segments were grown to different lengths. See Figure 3. The image was obtained using an optical microscope in reflected light mode, using a 400 ± 40 nm bandpass filter to improve resolution and enhance image contrast.

A80 cont'd

Page 34, line 28 to page 35, line 7:

The ability to place two different chemistries on the same Au/Pt nanobar code may also be used in this embodiment of the invention. This leads to two opportunities, self-referencing nanobar codes and dual-assay nanobar codes. Both opportunities stem from the fact that since it is possible to control which sets of stripes are within a single nanobar code, it is possible to control the position(s) on the particle from which fluorescence is observed. If, for example, a capture antibody is placed solely on the Au stripes and a sandwich immunoassay is carried out using a fluorescently-tagged secondary antibody, there should be no fluorescence emanating from the Pt stripes: the Pt stripes are acting as an internal standard. Thus, any fluorescence coming from the Pt must be ascribed to non-specific binding, and can be digitally subtracted. This principle can be demonstrated using matched and mismatched capture oligonucleotides on the different metals. This becomes a self-referencing nanoparticle.

A9

Page 44, lines 24-29:

Cylindrically-shaped colloidal metal nanoparticles of the present invention will be used in which the metal composition can be alternated (e.g., Pt/Au/Pt/Au/Pt) along the length, and in which the metal segments can be length-tuned. These nanobar codes will serve as solid phase identity tags for the immunoassays to be developed. In a typical sandwich immunoassay, the capture antibody will be conjugated to a specific nanobar code and the corresponding detection antibody will be labeled with a fluorophore.

A10